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For Review Only

Public Health Goal for
SIMAZINE
In Drinking Water

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LIST OF CONTRIBUTORS

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PREFACE

Drinking Water Public Health Goals

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California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based solely on health effects without regard to cost impacts and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas

PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR SIMAZINE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) proposes a Public Health Goal (PHG) of 0.0004 mg/L (0.4 µg/L, or 0.4 ppb) for simazine in drinking water. Simazine is a widely used selective herbicide with a high potential to leach into ground water and run off into surface waters. It is readily absorbed from the gastrointestinal tract and excreted via urine (≥ 70 percent) and feces (≥ 20 percent). It is metabolized via stepwise oxidative P-450 dealkylation to mono and didealkylated metabolites without disrupting the triazine ring. The proposed PHG is based on mammary tumors (mammary gland carcinomas) observed in female Sprague-Dawley rats administered simazine in the diet at 0, 10, 100 or 1,000 ppm for 24 months. The cancer slope factor was derived by linear extrapolation from the lower 95 percent confidence limit on the dose associated with a 10 percent increased risk of cancer (LED_{10}). A similar mechanism of action is expected for tumor induction by all s-triazines. Simazine is a weak mutagen.

The oral LD_{50} of simazine in rats and mice is greater than 5,000 mg/kg and sheep are more sensitive to the toxic effects of simazine than the other experimental animals tested. Signs of toxicity in sheep include incoordination, tremor, weakness, cyanosis and clonic convulsions. In the 24-month Sprague-Dawley rat study, reduced body weight was observed in both male and female rats along with changes in hematological parameters such as reduced red blood cell, hemoglobin and hematocrit levels and an increase in platelet counts at the high dose level. The no-observed-adverse-effect-level (NOAEL) was 0.5 mg/kg based on reduced body weights at the 100 and 1,000 ppm dose levels in female rats. The calculated noncarcinogenic health-protective concentration is 4 ppb based on the assumption that a 70 kg adult person consumes two liters of water per day and that the simazine contribution from drinking water is 20 percent. An uncertainty factor of 100 to account for inter- and intra-species variability was used in deriving this noncarcinogenic health-protective concentration.

The majority of the toxicity studies for hormonal effects were conducted on atrazine, a prototype congener for the s-triazines. Overall the data suggest that atrazine disrupts the estrous cycle and induces the growth of mammary tumors in test animals. It binds weakly to the estrogen receptor, alters a few estrogen-mediated parameters and displays no direct agonist or antagonist activity. Atrazine decreases triiodothyronine (T_3) levels in rats and causes thyroid hyperplasia. At high doses (≥ 40 mg/kg-day), it reduces luteinizing hormone (LH), progesterone and estradiol (E2) levels suggesting a hypthalamus-pituitary control.

The Maximum Contaminant Level (MCL) for both California and the U.S. Environmental Protection Agency (U.S. EPA) is 4 ppb.

INTRODUCTION

Simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) is a selective pre-and post-emergence herbicide for control of broad leaf and grassy weeds in various crops such as corn, almonds, grapes and oranges, and in

non-cropped area such as rights-of-way. Simazine inhibits photosynthesis. Average rates of 1 to 2.5 pounds per acre are usually applied by ground boom application, but higher doses may be used in nonselective conditions. At present only one dry flowable and two flowable liquid formulations are registered in California. Presently, atrazine and simazine are under "Special Review" by U.S. EPA because of potential carcinogenic risk from these s-triazine pesticides.

For the development of a PHG for simazine, two types of data were reviewed: data published in the open literature for the past ten years and data submitted to the California Environmental Protection Agency's Department of Pesticide Regulation (DPR) for the registration of simazine as a pesticide. The latter was initially reviewed by DPR (DPR, 1993) and brief summaries of the relevant toxicity data are provided in this document.

CHEMICAL PROFILE

Chemical names for simazine include: 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine which is also written as 6-chloro-N,N 1-diethyl, 1,3,5-triazine-2,4-diamine. Its trade name is Cekusima, Totazina. The chemical formula for simazine is $C_7H_{12}C1N_5$ and the chemical structure is presented in Figure 1.

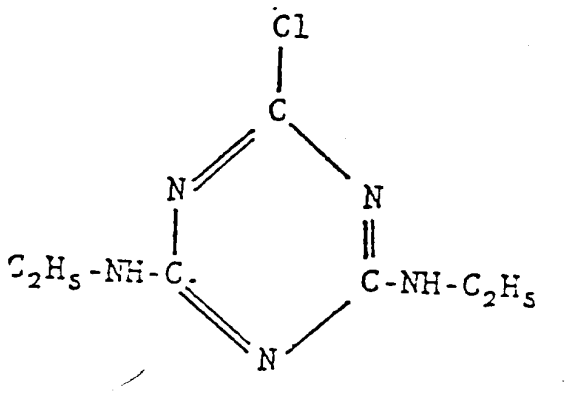

Figure 1. Simazine structure (DHS, 1988).

Production and Uses

Simazine is a selective pre-emergence herbicide for control of most annual grasses and broadleaf weeds. It is also used as an algaecide for aquatic weed control. It can also be formulated with other herbicides and fertilizers. At higher rates of application, it is used for nonselective weed control in industrial areas and along highways. The annual U.S. production of simazine was reported to be five million pounds in 1975 (NAS, 1977). In California, a total of 847,742 pounds of simazine was used in 1995 mainly on almonds, avocado, grapes, orange, olive, walnuts, lemons and rights-of-way (DPR, 1995). Simazine formulations are available as wettable powders, water dispersible granules, and in a liquid and granular form.

In 1984, U.S. EPA issued a registration standard for pesticide products containing triazines (atrazine, simazine and cyanazine) because of potential ground water contamination. Cyanazine has since been withdrawn voluntarily by the registrant. Atrazine and simazine are still in use. Both induce mammary tumors in rats and are classified by U.S. EPA as Group C, possible human carcinogens. Both are metabolized to similar degradation products, are similar in environmental fate and may be used as alternatives to each other. Atrazine is a prototype congener for the s-triazines and, therefore, the majority of the toxicity studies available are for atrazine. This health risk assessment on simazine draws on the available literature on atrazine in understanding the toxicity and its basis. OEHHA has recently completed a risk assessment of atrazine in support of a PHG for the chemical (OEHHA, 1998).

Physical and Chemical Properties

Important physical and chemical properties of simazine are provided in Table 1.

Table 1. Physical and chemical properties of simazine.

Property	**Value or Information**
Molecular weight	201.69
Color	white crystalline
Physical state	solid
Melting point	225 to 227°C
Water solubility	3.5 ppm at 20°C
Organic solvent	soluble in polar solvent
Solubility	low solubility in lipophilic solvent
Density	1.302 g/cm^3
Partition coefficients	1.7 @ 21°C
Log K_{ow}	1.94 to 2.26
Log K_{oc}	2.14
Vapor pressure	$6.1x10^{-9}$ mm Hg @ 20°C
Henry's law constant	$3.4x10^{-9}$ at mm^3/mol @ 20°C (calculated)

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Simazine has low water solubility and low vapor pressure. Therefore, it is not expected to be significantly volatile. However, simazine may be released into the environment as a result of manufacturing and through herbicide use.

Soil

Simazine is moderately persistent in soil. At the application rate of two pounds per acre and with favorable growth of microorganisms, soil sample analysis revealed an increased degradation rate of simazine. About 15 percent of the parent compound remained at one year after initial application and no residues could be detected after 16 months (Talbert and Fletchall, 1965). Simazine is more persistent in soils than atrazine and other triazines such as propazine, ipazine and chlorazine (Beatley, 1964; Switzer and Rause, 1964).

Under anaerobic conditions, [^{14}C] simazine was reported to degrade in loamy sand soil with half times ($t_{1/2}$) of 8 to 12 weeks (Keller, 1978). Degradation products included 2-chloro-4,6-bis(amino)-s-triazine, 2-hydroxy-4,6-bis(ethylamino)-s-triazine and 2-hydroxy-4-ethylamino-6-amino-s-triazine. Adsorption to soils is effected by the content of organic matter in the soil, the clay content and with soil action exchange capacity (Helling, 1971; Helling and Turner, 1968; Talbert and Fletchall, 1965). Monsanto (cited by U.S. EPA, 1987) reported $t_{1/2}$ for decomposition of 36 and 234 days for simazine in loamy sand and silt loam soils, respectively. Simazine decomposition in soils is effected by moisture content and temperature (Walker, 1976). The rate of simazine decomposition in soil is inversely affected by soil pH. Photodegradation and volatilization do not contribute significantly to this process. Simazine is hydrolyzed by a non-biological reaction to form a nonphytotoxic metabolite, hydroxysimazine. Dealkylation by free radical reactions is also involved in simazine environmental degradation. Soil persistence of simazine makes it useful in long-term weed control programs. Usually crops can be planted one year after simazine application at the selective rates (two to four pounds per acre).

In a series of unpublished studies, Martin et al. (1975) and Mattson et al. (1969) detected 2-chloro-4-ethylamino-6-amino-s-triazine, a degradation product of simazine, in soil up to 12 inches deep after simazine application in field studies. In the field, simazine $t_{1/2}$ was reported to be 30 to 139 days (Walker, 1976; Martin et al., 1975; Mattson et al., 1969).

Water

U.S. EPA has analyzed data on triazine pesticides detected in raw and finished surface water primarily in the 12–state mid-western corn belt region of the U.S. where the majority of the annual triazine use occurs (U.S. EPA, 1994). These data include results from field monitoring studies and literature reviews, and data submitted under the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Atrazine, simazine and certain degradation products were frequently detected in the same water samples, reflecting the pattern of herbicide usage in the studied watersheds. Atrazine and simazine follow similar degradation pathways. Both parent compounds form hydroxyl analogues and dealkylated

chloro-degradation products. Two of these chloro-degradation products are identical for simazine and atrazine. Based on the environmental fate and toxicity of these compounds, the cumulative effect on the environment of the triazine herbicides is assumed to be additive.

Ground water monitoring data submitted by pesticide registrants, state government agencies and the U.S. Geological Survey (USGS), as well as data collected by U.S. EPA's National Pesticide Survey of Drinking Water Wells and the Office of Pesticide Programs (OPP) have also been analyzed by U.S. EPA (U.S. EPA, 1994). In OPP's database for pesticides in ground water, simazine was the eighth most prevalent pesticide in 19 out of 30 states in which samples were collected. Seven percent of the samples that contained residues exceeded the federal simazine MCL of 4 ppb.

Simazine was the most frequently detected pesticide in California's Well Inventory Database. Residues of simazine have been reported in 20 counties in California at concentrations ranging from 0.02 to 49.2 µg/L (U.S. EPA, 1994).

Food

Human exposure to simazine can also result from ingesting foods containing residues remaining in or on treated crops such as corn, nuts and fruits. In addition, dietary exposure may occur by consuming products from animals that feed on simazine-treated crops. The major contributors of simazine risk from dietary exposure are from oranges and apples (U.S. EPA, 1994). U.S. EPA estimated a total dietary exposure of 1.1×10^{-4} mg/kg-day. Tolerances for simazine residues in or on certain raw agricultural commodities have been established. The values range from 0.02 ppm in animal fat and meat through 0.25 ppm in fruits and grain to 15 ppm in grass forage (Code of Federal Regulations, 1984). Health Canada's estimate of theoretical maximum daily intake is 0.2 mg/kg-day based on residue tolerance limits (Health Canada, 1986).

METABOLISM AND PHARMACOKINETICS

Absorption

Animals

^{14}C-simazine was administered orally as a single dose to Sprague-Dawley rats (five/sex/group) at 0.5 or 200 mg/kg. A third group was administered 0.5 mg/kg-day unlabelled simazine for 14 days followed by a single dose (0.5 mg/kg) of ^{14}C-simazine. At the low dose level, 51 to 62 percent of the administered dose was eliminated in the urine and 12 percent was found in the tissues. At the high dose, 22 percent of the administered dose was found in the urine and 2 percent was found in the tissues. In the third group, 59 to 66 percent of the dose was eliminated in the urine and 8 percent was found in the tissues. This suggests that about 70 percent of the administered dose of simazine is absorbed (Orr and Simoneaux, 1986).

Distribution

Following the administration of a single oral dose of 0.5 or 200 mg/kg-day simazine to Sprague-Dawley rats, the highest concentration was observed in red blood cells (RBC) with lesser amounts in liver after

seven days. The RBC concentrations were 16.3 to 19.9 ppm at the high dose and 0.18 to 0.23 ppm at the low dose. Residue levels were slightly higher in male rats than female rats. Low levels of simazine ranging from 0.0 to 0.16 ppm were detected in the heart, lung, spleen, kidney, liver, brain, plasma and bone (Orr and Simoneaux, 1986).

Metabolism

White female rats were administered a single oral dose of 0.5 mg/kg of ^{14}C ring labeled simazine (Simoneaux and Sy, 1971). Metabolites were analyzed in 24-hour collected urine by thin-layer chromatography and electrophoresis. The metabolites identified were hydroxysimazine, 2-hydroxy-4-amino-6-ethylamino-s-triazine and 2-hydroxy-4,6-diamino-s-triazine. These metabolites accounted for 6.8, 6.1 and 14.0 percent of the administered radioactivity, respectively. Approximately 50 percent of the radioactivity in urine was not identified (Simoneaux and Sy, 1971).

Charles River rats (male, 100 grams) were administered by gavage 1.0 mL of peanut oil containing 0.005, 0.55 or 50 mg/mL simazine (equal to 0, 0.017, 1.7, 17 or 167 mg/kg-day) twice in 24 hours. Urine samples (24-hour intervals) were analyzed by gas chromatography for the presence of mono and di-N-dealkylated metabolites. The di-N-dealkylated metabolites) 2-chloro-4,6-diamino-s-triazine) appeared to be the major product, ranging from 1.6 percent at 0.5 mg dose to 18.2 percent at the 50 mg/dose, while mono-N-dealkylated metabolites ranged from 0.35 percent at the 0.5 mg dose to 2.8 percent at the 50 mg dose. This suggests that the proportion of amount excreted is dose-dependent (Bradway and Moseman, 1982).

Excretion

Male and female Sprague-Dawley rats administered 0.5 mg/kg of ^{14}C simazine by the oral route excreted 50.5 and 62.5 percent of the administered dose, respectively, in the urine and 19.1 and 13.3 percent in feces, seven days after dosing. In rats given 0.5 mg/kg of simazine orally for 14 days followed by a single dose of ^{14}C simazine, male and female rats eliminated 58.5 and 66.0 percent of the administered dose in urine and 24.5 and 17.8 percent in feces, respectively. Rats given 200 mg/kg of ^{14}C-simazine orally excreted about 21 percent of the dose in the urine and about 2.0 percent in the feces (Orr and Simoneaux, 1986 cited by U.S. EPA, 1990).

Simazine residues were reported in the urine of sheep for up to 12 days following a single oral dose of 250, 500, 630, 700 or 1,000 mg simazine/kg (50 percent active ingredient). Concentration ranged from 6 ppm (low dose) to 70 ppm (high dose) from 2 to 10 days following administration of simazine (Hapke, 1968 cited by U.S. EPA, 1990).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

See Table 2 for a summary of acute toxicity data for simazine in laboratory animals. The oral LD_{50} of simazine in the rat and mouse is greater than 5,000 mg/kg and the inhalation LC_{50} in the rabbit is greater than 4.28 mg·hr/L. In primary eye irritation studies in rabbits, the U.S. Department of Agriculture (USDA, 1984) reported that simazine given at 71 mg/kg caused transient inflammation of the conjunctivae. The National Academy of Science (1977) listed the acute dermal lethal dose as more than 8 g/kg. The reported toxic effects following acute exposure were: chromorhinorrhea, chromodacryorrhea, perineal and/or abdominal staining, reduced activity, emaciation and ataxia before death.

Table 2. Summary of acute toxicity data for simazine in laboratory animals.

Species	Administration Mode	LD_{50}	References
Rat	Single oral dose	> 5,000 mg/kg	Martin and Worthington, 1977
Mouse	Single oral dose	> 5,000 mg/kg	USDA, 1984
Rabbit	Single oral dose	> 5,000 mg/kg	USDA, 1984
Rabbit	Inhalation (4-hr)	>4.28 mg·hr/L	Foster, 1994
Rabbit	Primary skin irritation, open	500 mg caused mild effect	Ciba-Geigy, 1977
Rabbit	Primary eye irritation	71 mg/kg caused transient inflammation of conjunctivae	USDA, 1984
Rabbit	Primary eye irritation	80 mg caused moderate irritant effect	Ciba-Geigy, 1977

Subchronic Toxicity

Sheep and cattle appear to be more susceptible to poisoning by simazine than most common laboratory animals. Repeated administration of simazine to sheep resulted in death after a total dose of 1,400 mg/kg body weight (bw) (100 mg x 14 times) given by drench application or 1,000 mg/kg bw (100 mg x 10 times) orally administered in capsules (Palmer and Radeleff, 1969). In another study (Hapke, 1968) it was shown that a single oral dose of 500 mg/kg bw was fatal to sheep within 5 to 16 days after ingestion. The animals that survived the exposure were symptomatic for two to four weeks after treatment and showed loss of appetite, increased water consumption, incoordination, tremor and weakness. Some of the sheep were cyanotic and clonic convulsions followed.

Cattle administered seven oral doses of 100 mg simazine/kg bw-day became moribund and were eventually sacrificed. Gross necropsy showed congestion of lungs and kidneys, swollen, friable livers and small hemorrhagic spots on the surface of the lining of the heart. Smaller doses were associated with transient body weight depression, depending upon the dosing schedule (Palmer and Radeleff, 1969).

Simazine given by gastric tube to rats at the rate of 15 mg/kg-day produced degeneration of an occasional hepatocyte during the first three days. Liver samples were taken under ether anesthesia from animals treated 3 and 28 days, 24 hours and 14 days after the last dose. The condition did not progress and a process of adaptation was observed (Oledzka-Slotwinska, 1974).

Simazine (98.1 percent) was administered in the diet of Charles River albino mice for 28 days at concentrations of 0, 10, 30, 300, 1,000 or 4,500 (Equal to: 0. 1.5, 4.5, 15, 45, 150, 450, 1,500 and 4,500 mg/kg-day based on the standard daily feed consumption rate of 15 percent of the body weight). The no-observed-adverse-effect-level (NOAEL) was 450 mg/kg-day based on tremor, generalized weakness and marked reduction in body weights and deaths at the two high doses of 1,500 and 4,500 mg/kg-day (Ciba-Geigy, 1976).

Simazine was fed in the diet to 10 Sprague-Dawley rats/sex/group at concentrations of 0, 200, 2,000 or 4,000 ppm (equal to 0, 10, 100 and 200 mg/kg-day based on the standard feed consumption rate of 5 percent of bw) for 13 weeks (Tai et al., 1985a). Hematological and clinical chemistry parameters and urinalysis were reported for blood and urine samples collected from animals prior to study termination. All animals were necropsied and tissues were examined histopathologically. Significant dose-related reductions in feed intake, mean body weight and weight gain occurred in all treated groups. At 13 weeks, various dose-related clinical effects were noted. These included changes in hematological parameters (decreased mean erythrocyte and leukocyte counts and increased neutrophil and platelet counts), clinical chemistry parameters (decreased mean concentrations of blood glucose, sodium, calcium, blood urea nitrogen (BUN), lactic dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT) and creatinine with increased cholesterol and inorganic phosphate concentrations) and urinalysis determinations (elevated ketone levels and decreased protein levels). Relative and absolute weights of adrenal, brain, heart, kidney, liver, testes and spleen were increased and ovary and heart weights decreased. Necropsies revealed no gross lesions attributable to simazine ingestion. A dose-related incidence of renal calculi and renal epithelial hyperplasia were detected microscopically in treated rats, primarily in the renal pelvic lumen, but only rarely in the renal tubules. Microscopic examinations revealed no other lesions that could be attributed to simazine ingestion. The majority of the alterations in clinical chemistry values may have been related to reduced feed consumption. Since these dietary levels of simazine seriously affected the nutritional status of treated rats, the results of this study are of limited value (Tai et al., 1985a).

Simazine (97.5 percent) was administered to seven to eight month old beagles (four/sex) for 13 weeks at concentrations of 0, 200, 2,000 or 4,000 ppm (equal to 0, 5, 50 and 100 mg/kg-day) (Tai et al., 1985b). All dogs were observed daily for signs of toxicity. Blood and urine were collected from all dogs at pre-dose and at days 44 and 92 of the study for hematological, clinical chemistry and urinalysis determinations. At the end of the study all dogs were necropsied for gross and histopathological examination. In the 4,000 ppm dose group, alopecia, tremor, dermatitis (males) and emesis were observed. Similar to the rat study, reduced feed consumption affected the nutritional status of the animals and hence reduced the value of this study. Due to the seriously affected nutritional status of the test animals, the results of this study were also considered of limited value (Tai et al., 1985b).

Simazine (50 percent active ingredient) was administered orally to 21 sheep at 0, 1.4, 3.0, 6.0, 25, 50, 100 or 250 mg/kg-day for various time durations up to about 22 weeks (Dshurov, 1977). Fatty and granular liver degeneration, diffuse granular kidney degeneration, neuronophagia, diffuse glial proliferation and degeneration of ganglion cells in the cerebrum and medulla were found. In sheep that died, spongy degeneration, hyperemia and edema were observed in the cerebrum; the degree of severity was related to the dose of simazine and the duration of exposure. The thyroid showed hypofunction after daily doses in the range of 1.4 to 6.0 mg/kg given for periods of 63 to 142 days. The most severe antithyroid effect followed one or two oral doses of 250 mg/kg, which in one sheep produced parenchymatous goiter and a papillary adenoma. Parenchymatous goiter was also seen in sheep administered simazine orally at 50 or 100 mg/kg once per week for approximately 22 weeks. Based on these data, a lowest-observed-adverse-effect-level (LOAEL) of 1.4 mg/kg can be identified. However, it is not clear from the study details whether the authors considered the 50 percent formulation when providing the dosage levels (Dshurov, 1979 cited by U.S. EPA, 1990).

In a 21-day subacute dermal toxicity study in rabbits, Ciba-Geigy (1980) reported that 15 dermal applications of technical simazine at doses up to 1 g/kg produced no systemic toxicity or any dose-related alterations of the skin.

Chronic/Carcinogenicity Studies

Rat

Simazine (96.9 percent) was administered in the diet to Crl:CD Sprague-Dawley rats at 0 (90/sex), 10, 100 (80/sex) or 1,000 ppm (90/sex) for 104 weeks (Ciba-Geigy, 1988). The NOAEL for this study was 10 ppm (equal to 0.5 mg/kg) based on decreased body weights at the 100 and 1,000 ppm dose levels in females. Also, decreased body weight gains and feed consumption were observed in both sexes at the 1,000 ppm dose level. The mortality rate was high for all groups, but was significantly different for female rats only at the 100 ppm dose level as compared to controls. A decrease in RBC, hemoglobin (Hb) and hematocrit (HCT) was observed in females in the 1,000 ppm dose group. In male rats, there was an increase in relative weights of the brain, liver, testes/epididymis, and a decrease in heart and relative heart weights at the 1,000 ppm. In female rats, there was a relative increase in brain, kidney and liver weights at 1,000 ppm. The incidences of mammary carcinoma were significantly increased at the 100 and 1,000 ppm in females (Table 3). Also, the combined incidence of adenomas and carcinomas was significantly higher in the highest dose tested as compared to controls. A significantly higher incidence of cystic glandular hyperplasia was observed at the highest dose. An increased incidence of rare kidney tubular adenoma was also observed at the 1,000 ppm dose level (2/70).

Table 3. Summary of mammary lesions in female rats fed diets containing simazine for two years[1].

Histopathological lesions	Dose (ppm)			
	0	**10**	**100**	**1,000**
Adenoma[2]	2/70[3]	4/70	1/70	5/70
Carcinoma	14/70	13/70	19/70*[4]	35/70**[4]
Fibroadenoma	22/70	27/70	19/70	40/70**
Total tumors[5]	39/90	33/88	31/80	61/80
Cystic glandular hyperplasia	51/70	50/70	53/70	65/70**

[1]Adopted from U.S. EPA's review of the Ciba-Geigy study (1988).
[2]Interim sacrifice and recovery group are not included in the estimation of tumors.
[3]Number of animals with specified observation/total number of tissue examined.
[4]Indicate significance at $P<0.05$ and $P<0.01$.
[5]As reported by Stevens et al. (1994) including interim sacrifice and recovery group animals.

An adverse effect disclosure statement was submitted by Ciba-Geigy (July 24, 1992). In the letter it was stated that in June 1989, Ciba-Geigy initiated two new oncogenicity studies on simazine using female Sprague-Dawley rats derived from the F_{2b} generation of the rat reproduction study (DPR, 1993). These animals were exposed to simazine in utero and for 24 months post-partum at dietary levels of 0, 10, 100 or

500 ppm. In addition, an age-matched group of control Sprague-Dawley female rats was employed in the study. The following two separate studies were performed: Study 1: treated and control female rats were allowed to mate with untreated male rats, then delivered and nursed the pups through lactation day 21. Study II: animals in this group were treated the same as those in Study I, except they were not mated. The in-life portion of this study was completed in June of 1991. The results observed after histological examination are presented in Table 4.

The disclosure statement also stated that the incidence of ovarian tumors was not elevated in the combined study previously submitted and reviewed by DPR (DPR, 1993) in which animals were dosed up to 1,000 ppm. Therefore, the ovarian findings in the two studies described above constituted new potential adverse effect (DPR, 1993).

Mouse

Simazine (96.5 percent) was administered in the diet to Crl:CD 1 (ICR) BR mice at 0 (90/sex/group), 40, 1,000 (80/sex/group) or 4,000 (90/sex/group) ppm for 95 weeks (Ciba-Geigy, 1988). The NOAEL was 40 ppm (6 mg/kg-day) based on decrease in body weight gain and decreased feed and water consumption observed in both male and female mice at 1,000 and 4,000 ppm. There were transitory increases in brain weight, relative brain, liver and kidney weights in females at 1,000 and 4,000 ppm and relative adrenal and heart weights, and increase in relative lung and thyroid/parathyroid weights in female mice at 4,000 ppm. There was no oncogenic effect observed with simazine (DPR, 1993).

Table 4. Ovarian neoplasia/hyperplasia incidence in female Sprague-Dawley rats following simazine exposure.

	Feeding level (ppm)				
Lesions/ Tumors	**0[a]**	**0[b]**	**10**	**100**	**1,000**
Nulliparous females					
Hyperplasia (Sertoli cells)	12/50	9/25	0/50	21/50	31/50
Sertoliform Adenoma	0/50	0/50	0/50	1/50	5/50
Primiparous females					
Hyperplasia (Sertoli cells)	17/50	7/25	14/48	14/47	28/49
Sertoliform Adenoma	0/50	0/25	0/48	0/47	1/49

[a]The test and control groups were derived from the F_{2b} litter of the two generation reproduction study.

[b]This control group was comprised of age-matched Sprague-Dawley female rats obtained from Charles River laboratories.

Dogs

Simazine (96.5 percent) was administered in the diet for 52 weeks to four Beagles/sex/dose at concentrations of 0, 20, 100 or 1,250 ppm (equal to 0, 0.5, 2.5 and 31.3 mg/kg based on standard conversion factor of 0.025) (Ciba-Geigy, 1988). The NOAEL was 20 ppm (0.5 mg/kg) based on reduced body weight gain at 100 ppm and decreased RBC, Hb and HCT and increase in platelet counts (DPR, 1993).

In a second experiment, simazine (97.5 percent) was administered in the diet of four dogs/sex/group at 0, 200, 2,000 or 4,000 ppm for 13 weeks. The NOAEL was 200 ppm (5.0 mg/kg) based on reduced body weight and feed consumption at 2,000 and 4,000 ppm. No clear target organ was identified (DPR, 1993).

Genetic Toxicity

Simazine was found to be negative in most of the in vivo and in vitro test systems evaluating gene mutation, chromosomal aberration and DNA damage. Some of the tests are reviewed in this section.

Simazine has been tested in a variety of microbial mutagenicity assay systems. None of the data has shown simazine to cause mutagenic results in standard prokaryotic test systems. These include tests employing the following organisms: *Salmonella typhimurium* (Simmons et al., 1978; Commoner, 1976; Eisenbeis et al., 1981) *Escherichia coli* (Simmons et al., 1978; Fahring, 1974), *Bacillus subtilis* (Simmons et al., 1978), *Serratia marcescens* (Fahring, 1974) and *Saccharomyces cerevisiae* (Simmons et al., 1978).

Simazine (96.9 percent) was tested in the Ames test at 0 (vehicle = DMSO), 10, 25, 50, 100 or 250 µg/plate on *Salmonella typhimurium* strains: TA98, TAIOO, TA1535, TA1537 and TA1538 with and without rat liver S-9. No mutagenicity was observed with any tester strain at any dose level tested. Positive controls functioned as expected (Ciba-Geigy, 1988b as reviewed by DPR, 1993).

Simazine (99.6 percent) was administered in one oral dose (gavage) at various levels to eight mice (Tif: MAGF, SPF)/sex/group. Part I: Cells were harvested at 16, 24 and 48 hours for control (0.5 percent Carboxymethyl cellulose) and simazine (5,000 mg/kg-limit test). Part 2: Cells were harvested at 24 hours for control (0.5 percent CMC) and simazine (1,250, 2,500 or 5,000 mg/kg-limit test) treatments. One thousand polychromatic (PCE) and normochromatic erythrocytes (NCE) each were scored/animal (five/sex/group) for micronucleus assessment. The PCE/NCE ratio/animal was determined by counting 1,000 erythrocytes. There was no increase in the number of micronuclei in polychromatic erythrocytes relative to negative controls (DPR, 1993).

Simazine (99.6 percent) was used on primary cultures of human lymphocytes for three hours at 0 (vehicle, DMSO), 6.25, 12.5, 25, 50 or 100 µg/mL with and without activation to test for chromosomal aberrations. No increase in chromosomal aberrations was observed with simazine-treated cells when compared to controls. Positive controls functioned as expected (DPR, 1993).

Simazine (99.6 percent) was administered orally to six Chinese Hamsters/sex/group at 0, 1,250, 2,500 or 5,000 mg/kg. One thousand cells in each of three/sex/group were analyzed for micronuclei at 24 hours, only after second dose. If the effect on cell cycling is not known (report gives no indication), the

laboratory animals should be sacrificed over a 12 to 72 hour period to ensure the detection of micronuclei which are detectable at six and normally for 12 hours before and after the time of peak frequency. No information on PCE/NCE or mitotic index is given. No adverse effect was noted, but the study design was inadequate because of inadequate sampling time (DPR, 1993).

Simazine (96.9 percent) at concentrations of 0 (DMSO or culture medium), 1.57, 4.72, 14.17, 42.5, 85 or 170 µg/mL were assayed with primary cultures of rat hepatocytes. Treatment period was for 16 to 18 hours in both the original and confirmatory tests. Analysis was performed by autoradiography (three slides/dose, 50 cells were scored/slide). Simazine did not induce DNA damage to primary hepatocytes. Positive controls functioned as expected (DPR, 1993).

Simazine induced mutations in the sex-linked recessive lethal test employing the fruit fly *Drosophila melanogaster* (Valencia, 1981). In the study reported by Murnik and Nash (1977), simazine increased X-linked lethal when injected into male *Drosophila melanogaster*, but failed to do so when fed to larvae. Simazine also induced sister chromatid exchange in human lymphocytes and induced mutation in mouse lymphocytes (Clayton and Clayton, 1994 as cited by Micromedex, 1998).

Simazine has been shown to be positive (Simmons, 1978) and negative (Waters et al. 1982) for unscheduled DNA synthesis (UDS) in the human lung fibroblast UDS assay.

Simazine (99.6 percent) was tested for DNA repair in primary rat hepatocyte cultures exposed to 0, 0.4 2, 10 or 50 µg/mL for five hours in the presence of 3H-TdR. No increase in UDS as evidenced by grains/nucleus was reported (DPR, 1993). Similarly, no increase in UDS was observed in human fibroblast cultures treated with simazine (99.6 percent) at levels of 0, 0.2, 1, 5 and 25 µg/mL without activation for five hours.

Simazine failed to produce chromosomal effects as indicated by the following tests: sister-chromatid exchange (Waters et al., 1982), mouse micronucleus assay (Waters et al., 1982) and mouse dominant lethal assay (Ciba Geigy, 1977).

Riccio et al. (1981) tested 11 pesticides for genotoxic activity in two yeast assay systems utilizing the diploid strains D3 and D7 of *Saccharomyces cerevisiae* in the presence and in the absence of rat metabolic activation system. Simazine was negative for all genetic endpoints in both assay systems.

Simazine is mutagenic in plants. It induces chromosome breaks and aberrations and increases aneuploidy and polyploidy in barley seeds (Wuu and Grant, 1966; Wuu and Grant 1967; Stroev, 1970). Plewa et al. (1984) evaluated genotoxic properties of 11 herbicides and 13 combinations of commercial grade herbicides with *Salmonella typhimurium* and *Saccharomyces cerevisiae* directly and following plant and animal activation. Simazine was tested only in situ with the pollen waxy locus assay of Zea maize and it was positive. Simazine is a plant activated promutagen according to this paper.

A water-soluble extract from maize plants exposed to three s-triazine herbicides (atrazine, simazine and cyanazine) has been shown to be mutagenic in strain TA 100 of *Salmonella* (Means et al., 1988). No mutagenic activity was observed in any control plant extracts using either water or a variety of organic solvents. Gel permeation studies of the extracts suggests that the mutagen(s) are small molecules (< 1,000 MW).

Ghiazza et al. (1994) studied the effects of atrazine on sister-chromatid exchange (SCE) frequency in human lymphocytes following exposure to 0.001, 0.01 or 1 ppm. A significant increase in SCE was observed at 1 ppm (5.07 ± 1.19 compared to 3.51 ± 1.14 in control).

Biradar and Rayburn (1995a) studied the effects of atrazine, simazine and bentazon on chromosomal damage in Chinese hamster ovary (CHO) cells by flow cytometry. The cell cultures were exposed to atrazine, simazine or bentazon at concentrations of 0.014, 0.080 or 0.005 μM, respectively, for 48 hours. The authors suggested that U.S. EPA deemed these concentrations safe for drinking water. A known clastogen (Ara-C) was used as a reference for comparing the magnitude of chromosomal damage caused by herbicides. Measuring the coefficient of variation and percent chromosomes present in the larger chromosome distribution peaks assessed chromosomal damage. Exposure to atrazine increased the coefficient of variation of the largest chromosome distribution peak suggesting clastogenicity. The negative control, atrazine and Ara-C's coefficient of variations were 3.73, 3.93 and 4.18, respectively. Chromosomes exposed to both simazine and-bentazon did not exhibit chromosomal damage. Atrazine concentrations higher (0.023 to 0.092) than the contamination limits exhibited a true clastogenic nature like Ara-C (4.16 compared to 4.18 in positive controls).

Developmental Toxicity Studies

Rat

Simazine (98.2 percent) was administered by gavage to 25 mated (presence of sperm, day 0 of gestation) CR1 COBS CD (SD) (BR) rats/group at 0 (vehicle, 2.0 percent carboxymethylcellulose), 30, 300 or 600 mg/kg during days 6 to 15 of gestation (Ciba-Geigy, 1986). The maternal NOAEL was 30 mg/kg-day based on decreased weight gain and feed consumption at 300 and 600 mg/kg-day dose levels. The developmental NOAEL was 30 mg/kg-day based on lack of ossification in head, teeth, centrum/vertebra and rudimentary 14th rib at 300 and 600 mg/kg-day.

Pregnant rats were exposed daily to chloroform vapors or aerosols of ethylenethiourea, thimet, simazine or bromacil during days 7 to 14 of gestation (Dilley et al., 1977). The highest concentration of simazine was 317 ± 89 mg/m^3. All animals were sacrificed on day 20 of gestation and the dams and fetuses were examined for gross changes. Fetuses were fixed in Bouin's solution or alcohol and examined later for teratology. Simazine did not cause any prenatal changes. None of the compounds was teratogenic at the concentrations used and under these experimental conditions.

Rabbit

Simazine (97 percent) was administered by gavage to 19 rabbits/group at 0, 5, 75 or 200 mg/kg-day during days 7 to 19 of gestation (Ciba-Geigy, 1991). The maternal NOAEL was 5 mg/kg based on decreased weight gain, anorexia, nervous tremors at 75 and 200 mg/kg dose levels. The developmental NOAEL was 5 mg/kg based on late resorptions at 75 and 200 mg/kg and reduced fetal weight at the 200 mg/kg dose level. A decreased number of viable fetuses was observed at the mid- and high-dose levels, but these were thought to be due to maternal toxicity at these levels. The author suggested that at doses of 75 mg/kg-day and higher, simazine was toxic to fetuses and dams, but was not embryotoxic nor teratogenic.

Sheep

Necrotic changes in germinal epithelium of the testes, and disturbances in spermatogenesis were observed in sheep which were fed simazine (50 percent active ingredient) at concentrations of 6 mg/kg for 142 days

or 25 mg/kg for 37 to 111 days. As only 50 percent of the simazine used was pure, in addition to being ill-characterized, the study can not be utilized for the purpose of risk assessment (Dshurov, 1979 as cited by U.S. EPA, 1990).

Reproductive Toxicity

Simazine (96.9 percent) was administered in the diet to Sprague-Dawley rats (30/sex/group) at 0, 10, 100 or 500 ppm (equal to 0, 0.5, 5 and 25 mg/kg-day) for two generations (Epstein et al., 1991). The systemic parental NOAEL was identified to be 10 ppm based on decreased body weight gain and decreased feed consumption in both male and female rats of both generations at greater than or equal to 100 ppm. The sporadic decreased feed consumption noted at 100 ppm was not considered to be compound-related. The reproduction NOEL was greater than 500 ppm. There were no reproductive effects at any dose levels tested (DPR, 1993).

Hormonal effects

Ovarian hormones are known to play a role in the development of mammary tumors. In order to better understand the role and possible mechanism of ovarian hormones in simazine-induced mammary tumors, we have reviewed several of the studies conducted on a prototype s-triazine (atrazine) that were recently submitted in support of the registration of atrazine as an active ingredient (DPR, 1998). The effects of atrazine and other triazines on the estrous cycle, estrogen mediated parameter responses, estrogen receptor binding, hormonal induction and metabolism have been the subject of several studies. The following is a brief summary of the key studies as well as related studies found in the open literature.

Atrazine is used as a prototype for s-triazines and therefore a similar mechanism of action is expected for tumor induction by the simazine. This is further supported by similar effects observed with atrazine and simazine in many studies described under hormonal effects. Overall the data suggest that atrazine disrupts the estrous cycle and induces mammary tumors. It binds weakly to the estrogen receptor, alters a few estrogen-mediated parameters and has no direct agonist or antagonist activity. At the high dose level, it reduces LH and estradiol by influencing hypothalamus-pituitary control mechanism.

Atrazine was fed in the diet to Fischer 344 rats at levels of 0, 10, 70, 200 or 400 ppm and to Sprague-Dawley rats at levels of 0, 70 or 400 ppm (Wetzel et al., 1994). There were 70 female rats in each dose group. Ten rats per group were sacrificed at 1, 3, 9, 12, 15 and 18 months, and all remaining animals at 24 months to determine various parameters such as estrous cycle, plasma hormone levels and tumor profiles. Atrazine fed female Sprague-Dawley rats spent more days in estrous as compared to controls. This was statistically significant after 9 and 18 months in the 400 ppm dose group and after one and nine months in the 70 ppm dose group. Plasma estradiol concentrations were also significantly increased at three months in female rats fed 70 or 400 ppm atrazine. No effect was observed on estrous cycle or estradiol concentration in the Fischer 344 rats. In Sprague-Dawley rats, tumor latency for mammary and pituitary gland tumors during the first year was shortened at the 400 ppm level. The overall incidence, however, over the two years was similar to controls and not statistically significant. This may be due to a high background rate of these tumors in Sprague-Dawley rats. Body weight gain was also lower at the 400 ppm level. The results suggest a possible effect of atrazine on tumor induction.

In Fisher-344 rats, reduced body weight gain was observed in the 200 and 400 ppm dose groups. No other effects were reported. The incidences of mammary and pituitary tumors were comparable across groups

and no evidence of an effect on time-to-tumor was noted. Based on these findings the authors hypothesized that high-dose atrazine administration in Sprague-Dawley females is related to an acceleration of age-related endocrine changes leading to an earlier onset and/or increased incidence of mammary tumors. The authors further suggested that this is due to atrazine interference with normal estrous cycling thus promoting prolonged exposure to endogenous estrogen. It is noteworthy, however, that the estrous cycle was also prolonged at the 70 ppm dose level but there was no concomitant increase in or earlier onset of mammary tumors in this dose group (Wetzel et al., 1994). Results of this current review showed that the data do not support the conclusion drawn by the authors. Because of the high background tumor incidence and small number of animals used in this study, it is difficult to draw conclusions about the effects at low dose levels.

It has been hypothesized that mammary tumors in Sprague-Dawley rats induced by simazine, atrazine and other s-triazines develop as a result of endocrine-mediated effects (Stevens et al., 1994). The authors compiled data from previously conducted carcinogenicity studies to substantiate this hypothesis. Atrazine and simazine, and to a lesser extent propazine and terbuthylazine, have been shown to induce mammary tumors in female Sprague-Dawley rats. The 2-thiomethyl-s-triazines (ametryn, prometryn, terbutryn and 2- methoxy-s-triazines) indicated a weak to no induction of mammary tumors. Hormonal data were given only for simazine at week 104 of the study which indicated marked changes in hormonal profile, but the significant differences were observed only at the highest dose of 1,000 ppm. The reported values for various hormone levels in control and 1,000 ppm dose levels, respectively, were: estradiol, 12±6 compared to 2 ±1; prolactin, 29 ± 18 compared to 204 ± 147; progesterone, 39 ±26 compared to 11 ± 9; growth, 11±2 compared to 37 ± 16.

The effects of simazine, atrazine and the common metabolite diaminochlorotriazine (DDA) were studied on estrogen-mediated parameters using several rat uterine model systems (Tennant et al., 1994a). For the effect on uterine weight, ovariectomized Sprague-Dawley rats were orally administered up to 300 mg/kg-day of atrazine, simazine or DDA for one to three days. On days two and three, half of each group of rats (three or four) received estradiol by injection. Dose-related decreases in uterine wet weights were obtained in rats treated with estradiol and atrazine as compared with estradiol treated controls. No effect was observed on uterine wet weight with atrazine alone as compared with vehicle control. For thymidine uptake studies, immature females were given 0, 1, 10, 50, 100 or 300 mg/kg-day atrazine, simazine or DDA for two days. On day two, all animals received estradiol by injection. After 24 hours, all animals were killed and uterine slices were incubated with ^{3}H-thymidine. Thymidine incorporation into uterine slices was decreased at the 50, 100 and 300 mg/kg-day dose levels. For uterine progesterone receptor binding studies, ovariectomized rats were dosed for two consecutive days with 50 or 300 mg/kg-day of atrazine, simazine or DDA. Each dose was followed by subcutaneous injection of estradiol. Parallel groups were treated with 0 or 300 mg/kg-day of atrazine, simazine or DDA without estradiol. Net progesterone receptor binding was reduced significantly in high dose animals in the atrazine and simazine treated groups and non-significantly in the DDA treated group subjected to estradiol treatment as compared with estradiol treated group alone. The authors concluded that triazine displayed very low antagonistic potency against estradiol function and they postulated that atrazine may operate through cellular interactions unrelated to these hormone effects.

The effects of simazine, atrazine, or DDA were studied on the binding of estradiol to the rat uterine estrogen receptor (ER) (Tennant et al., 1994b). Under equilibrium conditions none of the three triazines competed against the binding of radiolabeled estradiol to the ER. In ovariectomized rats, a concentration of 300 mg/kg-day of atrazine, simazine or DDA for two days reduced ER binding capacity by

approximately 30 percent. The authors suggested that atrazine binds weakly to the ER and other molecular interactions may play a part of triazine effect on target tissues.

The effect of atrazine on ovarian function was studied in Long-Evans hooded (LE-hooded) and Sprague-Dawley rats (Cooper et al., 1996a). Atrazine was administered by gavage to females displaying regular four-day estrous cycles for 21 days at doses of 75, 150 and 300 mg/kg-day. In both strains, a dose of 75 mg/kg-day disrupted the four-day ovarian cycle; but no distinct alteration in ovarian function (i.e., irregular cycles but not persistent estrus or diestrus) was observed. At 150 mg/kg-day atrazine induced repetitive pseudopregnancies in females of both strains. At 300 mg/kg-day, repetitive pseudopregnancies were induced in the Sprague-Dawley females, but the ovaries of the LE-hooded female appeared regressed and the smear cytology was indicative of the anestrus condition. These data demonstrate that atrazine,and probably other s-triazines like simazine can disrupt ovarian function and bring about major changes in the endocrine profile of female rats.

Atrazine was administered orally to Crl:CD Sprague-Dawley BR female rats (90/group) for 28 to 31 days prior to ovariectomy and continued for an additional 10 days at 0, 2.5, 5, 40 or 200 mg/kg-day (Morseth, 1996 as reviewed by DPR, 1996). The rats were evaluated for variations in estrous cycle stages by vaginal smear analysis during weeks two to four of the treatment. Seven days after ovariectomy, rats were implanted with a silastic capsule designed to deliver estradiol levels ≥12 pg/mL. On the tenth day after ovariectomy, blood samples were taken at intervals (20 to 25 samples/interval) for assays of estradiol (to verify capsule implantation), luteinizing hormone (LH) and prolactin. Prior to ovariectomy, estrous cycling was disturbed, most remarkably by prolonged periods of diestrus at the 40 and at the 200 mg/kg-day. Ovariectomized rats provided with estradiol-releasing implants had a remarkable decrement in LH peak levels at both the 40 and especially at the 200 mg/kg-day dose levels, a possible delay in timing of prolactin peak levels. Data are consistent with the hypothesis that the primary toxic action of atrazine leads to delays of ovulation (hence prolonged estrus) by disturbing the releases of LH and prolactin surge. The author suggested that the data support a "threshold" however the wide spacing of dose levels and the high degree of variability in response do not allow a definitive conclusion from these results.

Atrazine (200 mg/kg intraperitoneal for three days) suppressed the estrogen-induced surge of LH and prolactin in ovariectomized rats (Cooper et al., 1996b). However, the pituitaries of atrazine treated rats did release LH in response to gonadotropin-releasing hormone (GnRH). Using this model, the authors reported a dose- and time-dependent disruption of pulsatile LH release in rats exposed to 0, 75, 150 and 300 mg/kg atrazine. The authors concluded that atrazine disrupts the central nervous system control of pituitary function.

Atrazine (97 percent) or DDA (97.4 percent) was administered to groups of 15 female Crl:CD®BR rats for at least two weeks at dose levels of 100, 200 or 300 mg/kg-day (Morseth, 1990 as reviewed by DPR, 1996). Initially the high dose level was 400 mg/kg-day, but this was reduced to 300 mg/kg-day for both test compounds on day four due to excessive toxicity. Two groups of 15 animals each served as controls: one received only corn starch suspension vehicle and the other (a positive control for prolactin secretion) received an intraperitoneal dose of metoclopramide 20 minutes before sacrifice. Rats were sacrificed at the time of first determination of diestrous stage after at least 14 daily treatments, cycle stage being determined by vaginal cytology. Serum collected at sacrifice was assayed for prolactin, LH, FSH, progesterone and estradiol. The majority of atrazine treated rats at dose levels of 200 to 300 mg/kg-day as well as DDA treated rats at dose levels of 100 to 300 mg/kg-day had clinical signs of "thin" or "few or no feces" and a dose-related decrease in body weight. There was also a reduction in thymus weight in all groups. Coincident with the above signs of general toxicity, there were possible hormone level changes,

particularly decreases of LH, progesterone and estradiol in the 200 to 300 mg/kg-day DDA treated rats. In general, the high variability evident in hormone levels, coupled with high general toxicity in groups appearing to manifest hormone level changes, makes this study of very limited value for assessing intrinsic effects of test articles on hormone control (DPR, 1996).

Estrogenic activities of simazine or atrazine were assessed using an environmental estrogen (estradiol) bioassay which consists of a Gal-human estrogen receptor chimerical construct (Gal-EGO) and a Gal regulated Luciferase reporter gene (17m5-G-Lucia) which have been integrated into HeLa cells. A dose-dependent induction in luciferase activity was observed following treatment of the cells with 17β-estradiol. No significant induction was observed in reporter gene activity following treatment with chloro-s-triazines, suggesting that chloro-s-triazines do not interact with the ER (Balaguer et al., 1996).

The effects of simazine and atrazine were studied on estrogen receptor-mediated responses following in vivo and in vitro exposure (Connor et al., 1996). After exposure for three days to atrazine at 50, 150 or 300 mg/kg-day, uterine wet weights, progesterone receptor (PR) binding activity and uterine peroxidase activity were measured. No treatment-related effect was observed on any of the parameters studied. However, both compounds inhibited basal cytosolic PR binding and uterine peroxidase activity in a dose-independent fashion. For in vitro responses, cell proliferation and gene expression were measured in the MCF-7 human breast cancer cell line and the growth was measured in the estrogen-dependent recombinant yeast strain PL3, which requires the presence of an estrogen substance and functional ERs in order to grow on selective media. No effects were observed on basal or estradiol induced MCF-7 cell proliferation or on the formation of the PR-nuclear complex. No agonist or antagonist effects were observed on estradiol induced luciferase activity. The estrogen-dependent PL3 yeast strain did not grow on minimal media supplemented with atrazine or simazine in place of estradiol. The authors concluded that these results indicate that the ER does not mediate the estrogenic and antiestrogenic effects elicited by these chemicals.

The effects of simazine, atrazine, atrazine deisopropyl, or cyanazine on ER-mediated responses were studied in yeast expressing the human estrogen receptor (hER) and an estrogen-sensitive reporter gene (β-galactosidase) (Tran et al., 1996). In the presence of an estradiol concentration (20 nM) that induced maximal reporter gene activity in yeast, atrazine did not inhibit reporter activity. However, all s-triazines decreased reporter activity in a dose-dependent manner in the presence of a submaximal concentration of estradiol (0.5 nM). The estradiol-dependent activity of a mutant hER lacking the amino terminus was not inhibited by atrazine in yeast. Competition binding assays indicated that the all s-triazines including simazine displaced radiolabeled estradiol from recombinant hER. These results suggest that the ability of s-triazines to inhibit estrogen receptor-mediated responses in yeast occurred through an interaction with hER and was dependent on the concentration of estradiol.

The effects of lindane, atrazine and prometyrene were studied on the formation of the ER complex (Tezak et al., 1992). For the in vivo studies, 21 days old rats were administered lindane at 3 or 6 mg/100g bw; atrazine at 3, 6, or 12 mg/100 g bw; prometryne at 12 mg/100 g bw for seven days. Animals were killed after 28 days for in vivo and in vitro studies. Both in vivo and in vitro, atrazine significantly inhibited the formation of the estradiol ER complex in rat uterus cytosol. The inhibition was non-competitive; atrazine decreased the number of binding sites but not the affinity of the ER for estradiol.

The influence of s-triazine compounds (atrazine, prometryne and de-ethylatrazine) was studied in vivo and in vitro on testosterone metabolism and binding of 5 alpha-dihydrotestosterone to its receptor in the rat prostate (Kniewald et al., 1995). Both atrazine and prometryne reduced 5 alpha-dihydrotestosterone (5α-

DHT) formation. In addition, both significantly decreased the number of binding sites for 5α-DHT on the receptor molecule following in vivo or in vitro exposure, but the Kd value was not changed. The authors suggested that the inhibition of the enzymatic activities responsible for testosterone conversion and steroid hormone-receptor complex formation was non-competitive and reversible and that s-triazine compounds acted as antiandrogens.

Kniewald et al. (1997) evaluated the appearance of immunoreactive cells for LH and follicle stimulating hormone (FSH) at the rat adenohypophysial (AH) level with and without atrazine treatment. Atrazine (120 mg/kg) was administered daily intraperitoneally for seven days to females and per 72 hours for 60 days to males. The authors state that exposure to atrazine induces sterility in rats expressed by permanent diestrous in females and significant changes in spermatogenesis in males as observed by electron microscopy. However, no data or references were provided to support this statement. The presence of LH and FSH reactive cells were determined on 6 μM sections from AH stained by avidin-biotin–peroxidase complex method and counter stained with hematoxylin. Atrazine treated males had 17 and 24 percent lower LH and FSH immunoreactive cells, respectively, as compared with controls. Atrazine treated females had same percentage of LH and FSH reactive cells in AH as controls in diestrous. The authors conclude that Lueitinizing releasing hormone (LHRH) from hypothalamus stimulate within AH synthesis of FSH more than LH and that this increased FSH is responsible for the absence of cycle in the female rats.

The effects of atrazine on androgen converting enzymes and protein synthesis were studied in male porcine pituitary adenohypophysial gland (Kniewald et al., 1994). The pituitaries were removed from six-month-old pigs castrated at the age of three months. Fresh tissues were incubated with ^{14}C-testosterone and enzyme activities responsible for testosterone conversion were measured and expressed as pg of steroid/mg tissue. Atrazine was added to the incubation mixture at various concentrations; atrazine at 0.175 μmol to 0.7 μmol significantly inhibited 5α-reductase, which converts testosterone to DHT (24 ± 2 pg/mg compared to 32 ± 3.1 pg/mg in control) and 17β-hydroxysteroid dehydrogenase which converts testosterone to 5α-androstane-3α, 17β-diol (A-diol) (66 ± 5.4 pg/mg compared to 109 ± 8.2 pg/mg in control). Atrazine also inhibited protein synthesis in pituitary cytosol. Electrophoresis analysis of cytosolic protein indicated that while other major bands remained unchanged the purified fraction of 28.2 kilodaltons (kD) was increased and was identified after gel filtration and 2-D SDS PAGE as prolactin. These results may suggest a possible mechanism for the effects of atrazine on androgen control of reproduction.

The effect of atrazine was studied on the thyroid gland in female albino rats administered atrazine orally at 0.2 LD_{50} for periods of 6 or 12 days (Kornilovskaya et al., 1996). At the termination of dosing, the anesthetized animals were killed and blood was drawn for the determination of serum triiodothyronine (T3) and thyroxin (T4). A dose-dependent decrease in serum T3 concentrations was observed in all the treatment groups (control: 0.57 $nmoL^{-1}$; atrazine for six days: 0.35 $nmoL^{-1}$; atrazine for 12 days: 0.21 $nmoL^{-1}$). No effects were observed on the concentration of thyroxine. Histologically, the thyroid epithelium featured small cuboidal cells and occasional structures of the follicle confluence within epitheliomas. There was also an increase in the number of follicle-building thyroid cells and follicular volume and a decrease in nuclear volume. The authors suggested that the observed thyroid hyperplasia might be due to the activation of the hypothalamus-pituitary axis due to a decrease in T3 levels (Kornilovskaya et al., 1996).

The ability of various xenobiotics including atrazine was examined for inhibiting the binding of [^{3}H] physiological ligand (present at a concentration of 7 nM) to the rabbit uterine cytosol androgen and estrogen receptors, to rat androgen-binding protein and to human sex hormone-binding globulin (hSHBG).

Atrazine caused a statistically significant inhibition of specific binding of [^{3}H]5α-DHT to the androgen receptor. The binding of [^{3}H]5α-DHT to rat androgen-binding protein was inhibited 40 percent by atrazine. There was no inhibitory effect of atrazine on the binding of 5α-DHT to hSHBG (Danzo, 1997).

The metabolism of estradiol, using a radiometric assay that measures the relative formation of 16-α-hydroxyestrone (16-alpha-OHE-1) and 2-hydroxyestrone (2-OHE-1) from specifically tritiated estradiol in (ER+) MCF-7 cells, was studied in the presence of various environmental xenobiotics (Bradlow et al., 1995). The ratio of 16-alpha-OHE-1/2-OHE-1 observed after treatment with the known rodent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) was compared with the ratio after treatment with DDT, atrazine, γ-benzene hexachloride, kepone, coplanar PCBs, endosulfans I and II, linoleic and eicosapentenoic acids and indole-3-carbinol (I3C). All pesticides tested including atrazine significantly increased the ratio of 16-alpha-OHE-1/2-OHE-1 metabolites to values comparable to or greater than those observed with DMBA treatment. In contrast, the antitumor agent I3C increased 2-OHE-1 formation and yielded ratios that were a third of those found in unexposed control cells and 1/10th of those found in DMBA-treated cells. The authors suggested that atrazine xenobiotics might increase the risk of breast cancer by altering the ratio of 16-alpha-OHE-1/2-OHE-1. Estradiol metabolite 2-hydroxyestrone (2-OHE-1) inhibits breast cell proliferation while 16-alpha-hydroxyestrone (16-alpha-OHE-1) enhances breast cell growth, increases unscheduled DNA synthesis increases oncogene and virus expression and increases anchorage-independent growth.

Effects on estrous cycle

Simazine was not specifically tested for effects on the estrous cycle, but the marked increase in incidence of mammary tumors, results much like those characteristic in the case of atrazine, is well documented. In Sprague-Dawley female rats, atrazine exposure at about 20 mg/kg-day dose level increased the percentage of days in estrous and increased incidence and earlier onset of mammary tumors. The estrous cycle was also prolonged at the 3.5 mg/kg-day dose level, but there was neither an increase in nor an earlier onset of mammary tumors in this dose group. In another study conducted in Long-Evans hooded (LE-hooded) and Sprague-Dawley rats, a 75 mg/kg dose level for 21 days disrupted the four-day ovarian cycle, but no distinct alteration occurred in ovarian function (i.e., irregular cycles but not persistent estrus or diestrus in both strains of rat). At the 150 mg/kg-day dose level, atrazine induced repetitive pseudopregnancies in females of both strains. At the 300 mg/kg-day dose level, repetitive pseudopregnancies were induced in the Sprague-Dawley females, but the ovaries of the LE-hooded female appeared regressed and the smear cytology was indicative of the anestrus condition. A recent study in rats showed that a dose of 20 mg/kg-day atrazine given to ovariectomized female Sprague Dawley rats did not increase or cause earlier onset of mammary tumors. The genotoxic carcinogens DMBA and MNU both induced an increased incidence and earlier onset of mammary tumors in this experimental model.

Effects on estrogen mediated parameters

The effects of simazine and other chloro s-triazines including the protype atrazine were studied on estrogen-mediated parameters following in vivo and in vitro exposure. In vivo, dose-related decreases in uterine wet weights were obtained in rats treated with estradiol and atrazine, but not with atrazine alone. In addition, thymidine incorporation in uterine slices was decreased at the 50, 100 and 300 mg/kg-day dose

levels. For in vitro responses, no effects were observed on basal or estradiol-induced MCF-7 cell proliferation or on the formation of the PR-nuclear complex formation. No agonist or antagonist effects were observed on estradiol-induced luciferase activity. The estrogen-dependent PL3 yeast strain did not grow on minimal media supplemented with atrazine or simazine in place of estradiol. In an estrogen (estradiol) bioassay wherein binding of ligand to ER regulated luciferase reporter gene (17m5-G-Lucia) activity, a dose-dependent induction in luciferase activity was observed following treatment of the cells with 17β-estradiol, but not with atrazine and simazine. In yeast expressing the human estrogen receptor (hER) and an estrogen-sensitive reporter gene (β-galactosidase), atrazine did not inhibit reporter gene activity. However, atrazine decreased reporter activity in a dose-dependent manner in the presence of a submaximal concentration of estradiol (0.5 nM).

Effects on receptor binding

Simazine has not been tested in receptor binding assays. However, competition binding assays indicated that atrazine displaced the radiolabeled estradiol from recombinant hER. Net progesterone receptor binding was reduced significantly in high dose animals in the atrazine treated groups subjected to estradiol treatment and non-significantly in estradiol-pretreatment rats. In the absence of estradiol treatment, lesser but nevertheless statistically significant reductions in progesterone receptor binding was observed. In another study, atrazine alone for one to three days inhibited basal cytosolic PR binding. In rat prostrate, atrazine inhibited 5 alpha-dihydrotestosterone (5α-DHT) formation and decreased the number of binding sites for 5α-DHT on the receptor molecule following in vivo or in vitro exposure to atrazine, but the Kd value was not changed. Atrazine also inhibited the binding of tritiated physiological ligands, [^{3}H]5α-DHT and ABP (40 percent) to androgen receptors. There was no inhibitory effect of atrazine on the binding of 5α-DHT to hSHBG in rat prostate.

Effects on hormone induction and metabolism

In determining the effects of chloro s-triazines on hormonal induction, simazine was not tested. However, the prototype s-triazine compound atrazine has been studied in this context. Reduced levels of LH, progesterone and estradiol were observed in a recent rat study. While the exact mechanism of hormonal disruption by atrazine is not known, the alteration in estrous cycling is considered to be due to the disruption in hypothalamic-pituitary regulation. Atrazine inhibited 5α-reductase in male porcine pituitary adenohypophysial, which converts testosterone to DHT and 17β-hydroxysteroid dehydrogenase, which, in turn, converts testosterone to 5α-androstane-3α, 17β-diol (A-diol). Atrazine also inhibited prolactin synthesis in pituitary cytosol. A dose-dependent decrease in serum T3 concentrations was observed in all the treatment groups. No effects were observed on the concentration of thyroxine. Atrazine increased the ratio 16-α-hydroxyestrone (16-alpha-OHE-1) and 2-hydroxyestrone (2-OHE-1) from specifically tritiated estradiol in (ER+) MCF-7 cells to values comparable to or greater than those observed with DMBA treatment. In contrast, the antitumor agent I3C increased 2-OHE-1 formation and yielded ratios that were a third of those found in unexposed control cells and one-tenth of those found in DMBA-treated cells.

Toxicological Effects in Humans

No cases of systemic human poisoning from simazine have been reported in the published literature. However, according to Soviet reports (Elizaiov, 1972; Yelizarov, 1977) there were 124 cases of contact dermatitis noticed among workers manufacturing simazine and propazine. Mild cases lasting three or four days involved pale pink erythema and slight edema. Serious cases lasting seven to ten days involved greater erythema and edema and a vesiculopapular reaction that sometime progressed to the production of bullae.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The most sensitive endpoint for noncarcinogenic effects is reduced body weight and body weight gain in female rats in a two-year feeding study (Ciba-Geigy, 1988a). The NOAEL for this effect is 10 ppm (equal to 0.5 mg/kg based on standard feed consumption rate of 5 percent of the body weight). At the next highest dose of 100 ppm, statistically significant lower mean body weight was observed at different time intervals throughout the study and at study termination when compared to controls. At the highest dose of 1,000 ppm, mean body weighs, RBC, Hb and HCT were reduced and white blood cells were increased in both male and female rats. Reduced body weights were also observed in the two-year mouse feeding study (NOAEL 6 mg/kg-day), one-year dog (NOAEL 0.5 mg/kg-day), in a rabbit developmental study (NOAEL 5 mg/kg-day) and in the two-generation rat reproduction study (NOAEL 0.5 mg/kg-day). The long-term rat study was selected for the calculation of the health-protective level based on noncarcinogenic effects.

Carcinogenic Effects

Possible Mode of Action

There was a statistically significant increase in the incidence of mammary carcinomas in Sprague-Dawley rats at the 100 and 1,000 ppm dose levels. A statistically significant increase was also observed in mammary fibroadenoma and cystic glandular hyperplasia at the 1,000 ppm dose levels. No evidence of carcinogenicity was observed in the mouse oncogenicity study. Simazine was negative in the majority of the mutagenic studies except for a weak positive result for induction of a sex-linked recessive lethal mutation in fruitflies and an increase in SCEs in human and mouse lymphocytes. Simazine is positive in many of the plant genotoxic studies. However, the significance of plant genotoxicity studies in humans is not known.

The exact mechanism of tumor induction by simazine is also not known, but recent evidence from atrazine studies, a prototype s-triazine derivative, suggests that an endocrine-mediated mechanism is involved. The mechanism is estrogen receptor (ER) independent and the alterations observed in the regulation of estrous cycling are due to disruption of hypothalamic-pituitary regulation of ovarian function. On the basis of a lack of clear mechanism of action and similarity in the toxic endpoint (mammary tumors) of all triazines, a

linear dose-response relationship from the LED_{10} point of departure is appropriate for this risk assessment. (The LED_{10} is the 95 percent lower confidence limit on the dose that gives a 10 percent tumor response.)

U.S. EPA follows a general procedure in deriving MCLGs for group C carcinogens in water. Either an RfD approach is used (as with a noncarcinogen) but an additional uncertainty factor of 1 to 10 (usually 10) is applied to account for the limited evidence of carcinogenicity, or a quantitative method (potency and low dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range. According to the proposed guidelines for carcinogenic risk assessment (U.S. EPA, 1996), the type of extrapolation employed for a given chemical is based on the data supporting linearity or non-linearity or a biologically based or case-specific model. When insufficient data are available supporting either approach, the default is to use a linear extrapolation.

For the non-linear approach, either a NOAEL or a LED_{10} value can be used with an appropriate uncertainty factor. For the linear approach either, q_1* value or a carcinogenic slope factor (CSF) as has been suggested in the proposed cancer guidelines can be used to determine PHG levels. Since the mechanism of action of simazine is unknown, OEHHA has adopted the default option of linearity for this chemical and has used cancer slope factor (CSF) and not the q_1*, as has been done traditionally for the carcinogenic compounds.

Estimation of Cancer Potency and LED_{10}

The most relevant data for estimating the LED_{10} are based on the increased incidence of mammary tumors in Sprague-Dawley rats (Ciba-Geigy, 1988a) which demonstrated a statistically significant increase in mammary tumors at the mid and high dose levels. The multistage model is fit to the carcinogenicity dose-response data and the 95 percent upper confidence limit on the linear term (q_1*) is calculated using the standard likelihood procedures as employed by U.S. EPA. An alternative potency estimate using a carcinogenicity slope factor (CSF) proposed by U.S. EPA in its 1996 proposed guidelines was also calculated by linear extrapolation below the LED_{10} dose. LED_{10} is the lower confidence limit on a dose associated with 10 percent extra risk. ED_{10} was calculated using the linearized multistage model and $(\text{body weight})^{3/4}$ interspecies scaling. A good fit criterion of $p > 0.05$ was adopted for the Chi-square test. The LED_{10} value and other potency estimates are given in Table 5. The earlier quantitative estimate by U.S. EPA using the linearized multistage model is $1.2 \times 10^{-1}(\text{mg/kg-day})^{-1}$. OEHHA's cancer potency estimate (q_1*) and CSF are 0.104 and 0.092 $(\text{mg/kg-day})^{-1}$, respectively. The LED_{10} value is 1.08 (mg/kg-day). The potency estimates and the LED_{10} value were calculated using Tox-Risk (version 3) software (K.S. Crump Division, Clement International Corp., Ruston, LA).

Table 5. LED_{10} and potency estimates for simazine based on rat mammary tumors.

Parameters	q_1* $(\text{mg/kg-d})^{-1}$	Chi-square	P	K	MLE_{10}[a] (mg/kg-d)	LED_{10}[a] (mg/kg-d)	CSF $(\text{mg/kg-d})^{-1}$	U.S. EPA q_1* $(\text{mg/kg-d})^{-1}$
Values	0.104	0.58	0.75	3	1.60	1.08	0.092	0.120

[a] MLE and LED are given as dietary concentration on a 100 percent food basis in Tox-Risk. They were converted to mg/kg-day assuming 1.5 kg diet/day for 70 kg human body weight.

CALCULATION OF PHG

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for a chemical in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{NOAEL/LOAEL \times BW \times RSC}{UF \times L/day}$$

where,

NOAEL/LOAEL = No-observed-adverse-effect-level or lowest-observed-adverse-effect-level.

BW = Adult body weight (a default of 70 kg for male, 60 kg for female, or 10 kg for a child).

RSC = Relative source contribution (defaults of 20, 40, and 80 percent).

UF = Uncertainty factors (typical defaults of a ten to account for inter-species extrapolation, a ten for uncertainty from the subchronic nature of the principal study and a ten for potentially sensitive human subpopulations).

W = Daily water consumption rate: 2 L/day for 60 to 70 kg adult, 1 L/day for ten kg child, higher values of liter equivalents (Leq/day) are used for volatile organic compounds to account for inhalation and dermal exposure through showering, flushing of toilets and other household uses of tap water.

This calculation is based on the assumption that a 70 kg adult person consumes two liters of water per day and that simazine contribution from water is 20 percent. For simazine, the NOAEL is 0.5 mg/kg-day for reduced body weight based on two year rat feeding study. A cumulative uncertainty factor of 1,000 is used which includes inter- (ten) and intra-species (ten) correction factors and an additional factor of ten for the carcinogenic potential of simazine.

$$C = \frac{0.50 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{1{,}000 \times 2 \text{ L/day}}$$

$$= \mathbf{0.0035 \text{ mg/L} = 3.5 \text{ ppb}}$$

Carcinogenic Effects

The following general equation for carcinogenic chemicals can be used to calculate the public health-protective concentration (C) for simazine in drinking water (in mg/L):

$$C = \frac{BW \times R}{q_1^* \text{ or } CSF \times L/day} = mg/L$$

where,

BW = Adult body weight (a default of 70 kg).

R = *De minimis* level for lifetime excess individual cancer risk (a default of 10^{-6}).

q_1* or CSF = Cancer slope factor, q_1* is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model and CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}). CSF = 10 percent/ LED_{10}. Both potency estimates are converted to human equivalent [in $(mg/kg\text{-}day)^{-1}$] using $BW^{3/4}$ scaling.

L/day = Daily volume of water consumed by an adult (a default of 2 L/day).

For simazine, q_1*, CSF and the LED_{10} values of 0.104 and 0.092 $(mg/kg\text{-}day)^{-1}$ and 1.08 mg/kg-day were calculated from the mammary tumor data in rats from the two-year dietary carcinogenicity study of Ciba-Geigy (1988a). The potency estimates q_1*, as used by convention by U.S. EPA, and CSF as recommended in the proposed cancer guidelines (1996), are used for comparative purposes. An RSC is not included in the calculation of a health-protective concentration for a carcinogen as the use of the low dose extrapolation is considered adequately health-protective without the additional source contribution.

$$\text{C using q1*} = \frac{1 \times 10^{-6} \times 70\ kg}{0.104\ (mg/kg\text{-}day)^{-1} \times 2\ L/day} = \mathbf{0.00034\ mg/L = 0.34\ ppb}$$

$$\text{C using CSF} = \frac{1 \times 10^{-6} \times 70\ kg}{0.092\ (mg/kg\text{-}day)^{-1} \times 2\ L/day} = \mathbf{0.00038\ mg/L = 0.38\ ppb}$$

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or noncarcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water, for preparing foods and beverages. It is also used for bathing or showering and in washing, flushing toilets and other household uses resulting in potential dermal and inhalation exposures.

The estimated health-protective simazine concentrations in drinking water calculated using various approaches are summarized in Table 6.

Table 6. Simazine concentrations based on various approaches.

Endpoints	Approach	Concentration (ppb)
Non-cancer	NOAEL[a]	3.5
Cancer	q1*[b]	0.34
Cancer	CSF[c]	0.38

[a]NOAEL, no-observed-adverse-effect level.
[b]q_1*, carcinogenic potency determined by the linearized multistage model.
[c]CSF, cancer slope factor calculated from the LED_{10} ($0.1/LED_{10}$).

There is a ten-fold difference in the calculated values using q_1* based on the cancer endpoint compared to using the NOAEL based on the non-cancer endpoint, reduced body weights in rats. Therefore, the proposed PHG is based on the more health-protective concentration calculated using the cancer endpoint

with a linear approach. OEHHA has chosen to use the linear approach using the CSF (and not the q_1*) because the value obtained is not dependent on the model. In the absence of a clear mechanism of action for mammary tumors in rats and their relevance to humans, a linear default is appropriate to use for simazine. OEHHA considers a concentration of 0.4 ppb (rounded) for simazine based on CSF calculated from the mammary tumor data in Sprague-Dawley rats (Ciba-Geigy, 1998) to be the most appropriate value for a PHG.

RISK CHARACTERIZATION

Simazine has produced mammary tumors in one species (rat) and one gender (female). This is consistent with findings of mammary tumors observed with other s-triazines. Simazine may be converted to nitrosamine in the gastrointestinal tract in the presence of nitrate commonly found in the water. However, the tumor profile induced by simazine is not similar to that of nitrosamines. At present, the mechanism of simazine toxicity is not known. Recently, a mechanism for mammary tumors in rats involving changes in endocrine function (and potentially a threshold) was proposed for atrazine, the prototype for all s-triazines (Thakur et al., 1999). Atrazine is not directly estrogenic. It decreases luteinizing (LH) hormone levels in rats suggesting a hypothalamus-pituitary level control for these hormones. The reduced decrease in T3 levels observed in rats also suggests involvement of hypothalamus-pituitary control.

In developing the PHG, we have used polynomial equation from the LMS model for fitting the mammary tumor incidence data from Sprague-Dawley rats in the observed range and to determine the 95 percent lower bound LED_{10}. From the $LED_{10,}$ a model-free linear low dose extrapolation was made. Graphical representation of the observed and expected response suggested a good fit. In the absence of specific data on scaling between rats and humans (i.e., relative concentrations or activities of the active material at the target site), the dose-response was converted to human equivalent based on body weight to the 3/4 power scaling. This is a source of uncertainty in the calculation of the PHG for simazine.

The PHG for simazine in drinking water is based on the parent simazine compound alone. However, simazine and its metabolite deethylsimazine have been detected in ground and surface waters. This metabolite is toxicologically similar to simazine. Therefore, the approach taken in the development of the PHG for simazine would likely underestimate the possible risk to humans.

The PHG is based on exposure to simazine from drinking water. Because of the physical and chemical properties of simazine, some, but probably an insignificant amount of inhalation and dermal exposure is expected from bathing and showering. There are no data on dermal absorption for simazine. A varied dose-dependent dermal absorption of 2 to 20 percent based on rat studies wherein high concentrations of atrazine (s-triazines prototype) in acetone or tetrahydrofuran was used (DPR, 1998) is not appropriate in estimating the dermal exposure to simazine from bathing and showering. Contact time to the material in water is variable, but less than in the rat studies.

The PHG of 0.4 ppb for simazine in drinking water is based on a *de minimis* cancer risk level of 10^{-6}. The concentrations in water corresponding to cancer risk levels of 10^{-5} and 10^{-4} are 3.8 and 38 ppb, respectively. These values may be an over or underestimation depending on the assumptions used in body weight scaling and low dose extrapolation methods. Given the lack of scientific data in support of the value used for one or the other parameter, a stochastic analysis was not done because the parameters are not well defined and the methodology has not been finalized.

OTHER REGULATORY STANDARDS

U.S. EPA's MCL for simazine is 4 µg/L (4 ppb). This MCL was calculated based on the RfD of 0.005 mg/kg-day derived from a NOAEL of 0.5 mg/kg-day for decreased body weight from a two-year oncogenicity study in rats. The California MCL is also 4 ppb and is based on the same oncogenicity study and approach used by U.S. EPA. This MCL was derived before the peer review committee for simazine, under the Office of Pesticide Programs, determined it to be a group C carcinogen and recommended that the caracinogenic risk be quantified. The Health Canada (1986) established an interim maximum acceptable concentration of 10 ppb for simazine in drinking water. This is based on a NOAEL of 5 mg/kg-day from a two-year dog study with an uncertainty factor of 4,000. OEHHA's PHG of 0.4 ppb is based on the cancer slope factor of 0.092 $(mg/kg\text{-}day)^{-1}$ derived from the mammary tumor data from Sprague-Dawley rats.

Simazine has not yet been considered for listing under Proposition 65. However, it is in the Proposition 65 prioritization tracking database for future consideration for listing as a chemical known to cause cancer or reproductive toxicity by the State's qualified experts (OEHHA, 1997).

Recently, U.S. EPA has used the linearized multi-stage model to extrapolate tumor responses observed at the high dose to predict tumor response at low doses. This model assumes that there is no threshold for carcinogenic effects. Based on this model, U.S. EPA calculated a cancer potency (q_1*) of 0.12 $(mg/kg\text{-}day)^{-1}$. The MCL of 4 ppb is associated with an estimated cancer risk level within the 10^{-5} range for drinking water (assuming a person consumes two liters of water per day containing simazine at 4 µg/L over a 70-year lifetime) (U.S. EPA, 1994).

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